



Microbial contamination level and microbial diversity of occupational environment in commercial and traditional dairy plants

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Abstract

Objective. The aim of this study was to assess microbial contamination of the air and surfaces at workplaces in commercial (CD) and traditional (TD) dairies.

Materials and method. Bioaerosol (impactor) and surface (swab) samples were collected in CD and in TD. Bacterial and fungal concentrations in the air and on surfaces were calculated and all isolated microorganisms taxonomically identified, based on their morphological, biochemical and molecular features.

Results. Average concentrations of bacterial aerosol ranged between 70–860 CFU/m³ and 265–14639 CFU/m³, while for fungal aerosol were between 50–290 CFU/m³ and 55–480 CFU/m³ in CD and TD, respectively. Average bacterial concentrations on surfaces ranged between 1.0–49.7 CFU/cm² and 0.2–60.4 CFU/cm², whereas average fungal surface contamination ranged between 0–2.7 CFU/cm² and 0–4.6 CFU/cm² in CD and TD, respectively. Qualitative analysis revealed mainly the presence of saprophytic microorganisms; however, several pathogenic strains (*Staphylococcus aureus*, *Streptococcus intermedius*, *Clostridium perfringens*, *Actinomyces* spp., *Streptomyces* spp., *Candida albicans*) were also isolated from both the air and surface samples in the studied dairies.

Conclusions. The air and surfaces in TD were more polluted than those in CD; however, in both types of dairies, the levels of microbial contaminants did not exceed respective threshold limit values. Nevertheless, the presence of pathogenic microorganisms may increase health risk for dairy workers and influence the quality of products. Hence, proper hygienic measures should be introduced and performed to guarantee high microbial quality of both production processes and milk products.

Key words

occupational exposure, bacteria, fungi, bioaerosols, surface swabs, dairy plants

INTRODUCTION

On a world scale, the dairy sector plays an important economic role in providing jobs for rural communities. According to the International Dairy Federation [1], the number of people employed in the dairy industry is about to 200,000 in Russia, 230,000 in China, and 500,000 in Egypt. In this branch of industry, the presence of microorganisms is an immanent part of many production processes. In the dairy industry, microorganisms can play both a positive role in the food production system and a negative one, causing contamination of the working environment.

The variety of factors that may affect food safety has led to the development of several management systems as Good Hygienic Practice (GHP), Good Manufacturing Practice (GMP), Hazard Analysis Critical Control Points (HACCP) system, etc. Their main task is to ensure that the resulting food product will be safe for the consumers [2]. Their use allows, among others, pollution control (including biological contaminants) for the ready-to-consume products and consumer safety, without neglecting the safety of the workers involved in the production processes.

Raw milk, even when milking is performed under aseptic conditions, is microbiologically contaminated. Microorganisms can enter the raw milk from the cowshed bedding, fodder, animal skin and hair and milking equipment, as well as via the clothes and hands of the workers [3]. Milk, being rich in nutrients such as proteins, lipids and sugars, provides an ideal environment for the growth of various microorganisms [4]. Natural milk microbiota is composed primarily of lactic acid bacteria (LAB); however, the presence of milk-borne bacteria and fungi responsible for human infections is also possible [5, 6]. The list of bacteria that can cause milk contamination is long and usually includes: *Campylobacter jejuni*, *Bacillus cereus*, *Escherichia coli* (including Shiga toxin strains), *Coxiella burnetii*, *Mycobacterium* spp., *Salmonella* spp. (including *Salmonella* Typhi), *Yersinia enterocolitica*, *Pseudomonas aeruginosa*, *Corynebacterium* spp., *Clostridium perfringens*, *Nocardia* spp. and *Staphylococcus aureus* strains, including those producing heat-stable toxins [4, 7]. Raw milk may also contain yeasts from *Candida*, *Cryptococcus*, *Pichia*, *Debaryomyces*, *Geotrichum*, *Kluyveromyces*, *Rhodotorula*, *Trichosporon*, *Saccharomyces*, and *Yarrowia* genera as well as molds from *Aspergillus*, *Chrysosporium*, *Cladosporium*, *Engyodontium*, *Fusarium*, *Penicillium*, and *Mucor* genera [8, 9, 10].

Although both the Food and Drug Administration (FDA) and Centers for Disease Control and Prevention (CDC) in

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the USA recommend the pasteurization of milk intended for human consumption [11, 12], many dairy products like cheese, butter or cream are still made from raw (unpasteurized) milk, especially in traditional dairies. As many consumers clearly demand and expect the food manufactured from 'high-quality raw materials' [13], this type of market pressure may result in increased risk for dairy workers [14, 15].

Dairy workers can be exposed not only to saprophytic and pathogenic microbiota from raw milk, but also to starter cultures of selected bacterial and fungal strains. Such cultures being added during milk processing support fermentation and/or food preservation processes lead to the improvement of texture, flavour, and nutritional value of dairy products [16]. The most popular starter cultures are lactic acid bacteria (LAB), such as *Lactococcus lactis*, *Lactobacillus delbrueckii*, *Leuconostoc mesenteroides* or *Streptococcus thermophilus*. There are also selected mould strains used for ripened cheese production, including *Penicillium roqueforti*, *Penicillium camemberti*, and *Penicillium candidum* [17, 18]. According to the FDA, starter cultures are clearly classified as Generally Recognized As Safe (GRAS) [11, 19]. However, GRAS status applies to the use in food production only, and information about health effects of airborne and dermal exposure to GRAS strains is still very scarce. So far, few publications have proved that workers from occupational areas with high levels of airborne LAB reported more health symptoms (e.g. irritations and immune system responses), compared to control groups [20, 21].

OBJECTIVE

Data regarding bioaerosols in dairy barns is available [22, 23]; however, information about microbial diversity and concentration in bioaerosols and on surfaces in the occupational environment of dairy plants are still scarce. Taking the above into account, the aim of this study was to perform the complex quantitative and qualitative characterization of microbiological agents in the air and on surfaces at workplaces in commercial and traditional dairies, to provide important information for proper occupational risk assessment and occupational risk management for workers in dairies.

MATERIALS AND METHOD

Environmental field samples. Bioaerosol and surface swab samples were collected in three commercial and three traditional dairy plants located in the central and eastern regions of Poland during summer 2018 (June–August). The examined dairy plants represented typical dairies of the sector of small and medium enterprises, and specialized in milk, butter, cream, yoghurt and cheese (excluding mould-ripened cheese) production. The samples were collected both in commercial dairies (CD) with an almost fully automated production process, and in traditional dairies (TD) where many production activities were manually performed. The buildings of each type of dairy plant were differentiated, taking into account the type and characteristics (size, type of ventilation), number of workers, methods of production (automatic, manual, mixed), nearby presence of animals (dairy cattle), and type of processed milk (raw, pasteurized).

Table 1 shows the buildings of commercial and traditional dairy plants with their detailed characteristic. To evaluate the potential influence of external microbial pollution sources on air quality at the studied workplaces, background air (atmospheric bioaerosol) samples were simultaneously collected in close vicinity (approximately 300 m from the border) of the studied dairies.

Table 1. Description of the commercial and traditional dairy plant buildings

Type of dairy plant Characteristic	Commercial	Traditional
Size	> 2,000 m ²	~ 200–300 m ²
Type of ventilation	mechanical	natural
Number of workers	160–250	2–10
Method of production	fully automated (excluding packaging)	manual or mixed (semi- automatic)
Nearby presence of animals (cattle)	no	yes (milk reception area in neighborhood with milking area)
Type of processed milk	pasteurized	raw

All samples were collected after obtaining the appropriate permits issued by CD and TD authorities.

Bioaerosol sampling. In the examined dairies, bioaerosol samples were collected during regular work hours at the following workplaces: milk reception, milk storage, cheese production, cream and butter production, and packaging area. Bioaerosol samples (100 L) were collected using properly calibrated MAS impactor (model 100-NT, Merck Eurolab GmbH, Darmstadt, Germany) on appropriate agar media: trypticase soy agar (TSA) with 5% of defibrinated sheep blood (bioMérieux, Marcy-l'Étoile, France) for mesophilic bacteria, MRS agar (agar De Man, Rogosa and Sharpe, bioMérieux) for lactic acid bacteria, Schaedleragar (SCS, bioMérieux), with 5% of defibrinated sheep blood for anaerobic bacteria, and malt-extract agar (MEA, Oxoid, UK) for fungi. All air samples were collected at the height of 1–1.5 m above the floor or ground simulating aspiration from the human breathing zone [24, 25].

During bioaerosol measurements, temperature and relative humidity of the air were measured with a portable thermohygrometer (model TFA 30.5024, Conrad Electronic GmbH, Hirschau, Germany).

Surface swab sampling. Surface samples of 100 cm² area (limited by the use of 10×10 cm sterile template) were collected with sterile swabs (Copan, Brescia, Italy) from worktops within the milk reception zone, surfaces of tanks within the milk storage area, worktops in the cheese production zone, worktops within the cream and butter production area, and from conveyer belts within the packaging area.

After shaking the swabs for 30 minutes at room temperature, a series of dilutions for each sample was prepared (10⁻¹–10⁻³) and used in triplicates in the amount of 0.1 mL for inoculation of proper medium (see above) for each examined group of microorganisms.

Laboratory analysis. Agar plates with bioaerosol and surfaces swab samples were incubated in the following conditions: 1 day (37 °C) + 3 days (22 °C) + 3 days (4 °C) for mesophilic

bacteria, 3 days (37 °C) in anaerobic condition for lactic acid bacteria, 3 days (37 °C) + 2 days (30 °C) in anaerobic condition for anaerobic bacteria and 4 days (30 °C) + 4 days (22 °C) for fungi [26]. The final concentration of microorganisms in bioaerosol was determined as colony forming units per 1 m³ of air (CFU/m³), while on surfaces as CFU per 100 cm² (CFU/100cm²).

All isolated microbial colonies were then classified to the genus and/or species level, taking into account their macroscopic and microscopic features. Bacterial and yeast strains identification was supplemented with proper biochemical (API) tests (bioMérieux). Filamentous fungi were identified based on their macro- and micro-morphology with proper taxonomic keys [27, 28, 29, 30]. The most prevalent microbial isolates were also analyzed by molecular polymerase chain reaction, followed by random amplification of polymorphic DNA method (RAPD-PCR) [31]. Bacterial and fungal DNA was isolated from pure cultures using Qiamp DNA Mini Kit (Qiagen, Hilden, Germany) for bacteria and Fungi DNA Mini Kit (Syngen Biotech, Wrocław, Poland) for molds and yeasts. The isolated bacterial DNA was amplified in PCR reactions with BAK11w (5'-AGTTTGATCMTGGCTCAG-3') and BAK2 (5'-GGACTACHAGGGTATCTAAT-3') primers for amplification of bacterial 16S rRNA gene fragment adequate to *E. coli* 16S rRNA gene positions from 10–806. In turn, the isolated fungal DNA was amplified in PCR reactions with ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') primers for amplification of fungal genome fragment located between 18S-28S rRNA genes of ITS1, 5.8S rRNA, and ITS2 fragments. PCR products were then purified and sequenced with ABI3730 Genetic Analyzer (Applied Biosystems, Waltham, USA). Comparison of DNA fragments to GeneBank database (National Center for Biotechnology Information, National Library of Medicine, USA) was performed using BLAST algorithm (Basic Local Alignment Search Tool) [32, 33, 34].

Statistical analysis. The obtained results were statistically analyzed with Kruskal-Wallis and Mann-Whitney tests using STATISTICA, version 7.1 (StatSoft Inc., Tulsa, USA). P values below 0.05 were considered statistically significant.

RESULTS

Quantitative analysis of bioaerosol samples. Average concentrations of bacterial and fungal aerosols in commercial and traditional dairies are shown in Table 2. Statistical analysis showed that bacterial aerosol concentrations were statistically higher at workplaces in TD than in CD ($p < 0.05$).

Table 2. Bacterial and fungal aerosol concentrations (CFU/m³) in commercial (CD) and traditional (TD) dairies

Type of dairy	Bacteria		Fungi	
	Median	Range	Median	Range
CD	265	40–980	165	40–390
TD	2345	200–26620	230	20–530
Atmospheric background near CD	165	90–130	1130	980–1280
Atmospheric background near TD	780	760–800	1280	1230–1330

At the same time, the concentration of bacterial aerosols in TD and CD were significantly higher than those measured in the atmospheric background near the studied dairies ($p < 0.05$). The concentration of fungal aerosols revealed an opposite relationships being significantly lower than those measured in the atmospheric background ($p < 0.05$).

Concentrations of bacterial and fungal aerosols at the studied workplaces in dairies are shown in Table 3. Average concentrations of bacteria in CD and TD ranged between 70–860 CFU/m³ and 265–14639 CFU/m³, respectively. Average concentrations of fungi in CD and TD ranged between 50–290 CFU/m³ and 55–480 CFU/m³, respectively. The highest concentrations of microbial aerosols in both types of studied dairies were detected within the milk reception zone. However, despite of existing variations in bioaerosol concentrations, the Kruskal-Wallis test did not show statistically significant differences between the studied workplaces ($p > 0.05$).

Within the tested processing areas, the air temperature ranged between 23.3–24.7 °C, while relative humidity between 34.9–55.1%. Neither air temperature, nor the relative humidity significantly influenced the bacterial and fungal aerosol concentrations ($p > 0.05$).

Qualitative analysis of bioaerosol samples. Percentage distributions of microbial groups identified in bioaerosols in the studied dairies and in the atmospheric air are shown in Figure 1. In bioaerosol collected in CD, non-sporing Gram-positive rods (including LABs) were predominant, accounting for 46.3% of total microbiota, followed by Gram-positive cocci (24.8%). Fungi, Gram-positive bacilli and mesophilic actinomycetes constituted 18.9%, 9.6% and 0.4% of the total microbiota, respectively. In TP, the most numerous were Gram-positive cocci (38.3%), followed by non-sporing Gram-positive rods (including LABs; 30.5%). Fungi, Gram-positive bacilli and mesophilic actinomycetes constituted 15.5%, 14.7% and 1.0% of the total microbiota, respectively. In the atmospheric air, fungi were the most abundant group of isolated microorganisms (55.5% of total isolated microbiota).

All bacterial and fungal strains isolated from the air of the studied dairies, with their percentages in relation to the total microbiota, are listed in Table 4. 18 bacterial species belonging to 9 genera were isolated in CD and 27 bacterial species belonging to 10 genera were isolated in TD. *Bifidobacterium* representatives were the most frequently isolated bacteria in CD (17.8%) and TD (15.6%), followed by *Lactobacillus paracasei* ssp. *paracasei* (9.7%) in CD and by *Streptococcus thermophilus* (11.5%) in TD. In case of fungi, 12 species belonging to 5 genera were isolated in CD and 19 species belonging to 8 genera were isolated in TD. In both types of dairies, the most frequently isolated moulds belonged to the *Penicillium* genus. Bioaerosol sampling also revealed the presence of yeast strains, among which the most frequently isolated was *Geotrichum candidum*, constituting 2.7% and 1.3% of isolated microbiota in CD and TD, respectively.

Biological agents from risk group 2 (according to the classifications in Directive 2000/54/EC and in Ordinance of the Polish Minister of Health) [35, 36] were also detected among isolated microorganisms. In CD, *Actinomyces* spp. constituted 0.4%, while in TD *Staphylococcus aureus* and *Streptomyces* spp. contributed to 4.5% and 1% of total microbiota.

Table 3. Concentrations of bacteria and fungi (CFU/m³) at the studied workplaces in commercial (CD) and traditional (TD) dairies

Workplace	Bacteria				Fungi			
	CD		TD		CD		TD	
	Median	Range	Median	Range	Median	Range	Median	Range
Milk reception	860	770–950	14639	2658–26620	290	190–390	480	430–530
Milk storage	680	380–980	2345	2190–2500	265	190–340	330	320–340
Cheese production	120	110–130	1940	1690–2190	50	30–70	95	90–100
Cream and butter production	70	40–100	2808	2820–2795	165	140–190	230	150–310
Packaging area	265	180–350	265	200–330	70	40–100	55	20–90

For the most frequently isolated species and for the microbial agents belonging to the risk group 2, identified using biochemical and microscopic techniques, the molecular analysis confirmed their sequence similarities at the level of 99–100%, with the sequences deposited in the GenBank database.

Quantitative analysis of surface samples. Concentrations of bacteria and fungi on surfaces in CD as well as TD ranged between 1.0–49.7 CFU/cm² and 0.2–60.4 CFU/cm², as well as between 0–2.7 CFU/cm² and 0–4.6 CFU/cm², respectively (Tab. 5). The highest concentrations of bacteria in CD and TD were observed on cheese production worktops (49.7 CFU/cm² and 60.4 CFU/cm², respectively), followed by worktops in milk reception and surfaces of tanks in milk storage. The highest concentrations of fungi in CD were observed on worktops in the cheese production area (2.7 CFU/cm²), while in TD on worktops in milk reception (4.6 CFU/cm²). There were no fungi detected on worktops in the cream and butter production area, or on surfaces from conveyor belts in packaging area in both CD and TD.

Qualitative analysis of surface samples. Percentage distribution of microbial groups identified on surfaces in the studied dairies is shown in Figure 1. On surfaces in both CD and TD, non-sporing Gram-positive rods (including LABs), as well as Gram-positive cocci, were predominant constituting 60.8% and 57.5%, as well as 30.4% and 28.3% of total microbiota, respectively. All bacterial and fungal species isolated from surfaces as well as their percentage contribution to the total microbiota are listed in Table 4. In the case of bacteria, 16 species belonging to 9 genera were isolated from surfaces at workplaces in CD, while 18 species belonging to 10 genera were isolated at workplaces in TD. The most frequently isolated bacterial strains in CD were *Lactobacillus delbrueckii* ssp. *delbrueckii* (20.5%), followed by *Bifidobacterium* spp. (17.1%), and *Lactobacillus fermentum* (13.2%). The most frequently isolated bacterial strains from surfaces in TD were *Lactobacillus curvatus* (18.5%), followed by *Lactobacillus paracasei* ssp. *paracasei* (14.3%), *Streptococcus thermophilus* (10.5%), and *Lactobacillus fermentum* (11.2%). In turn, 11 fungal species belonging to 9 genera were isolated from surfaces in CD, while 13 species belonging to 9 genera were

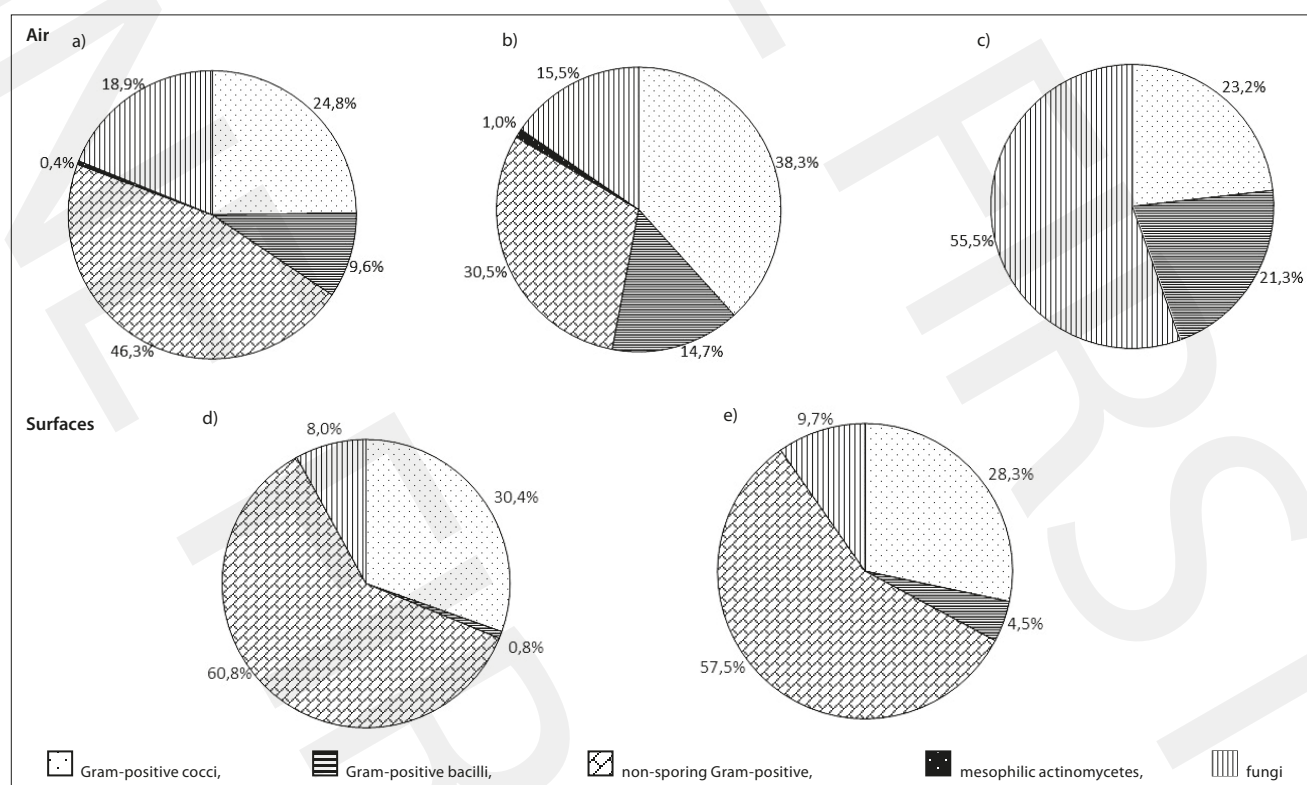
**Figure 1.** Percentage contribution of microbial groups to total microbiota isolated from the air (a–c) and surfaces (d–e) samples collected in commercial (CD: a, d) and traditional (TD: b, e) dairies as well as in atmospheric air (c)

Table 4. Percentage contribution (%) of bacteria and fungi to total microbiota isolated from the air and surface samples collected in commercial (CD) and traditional (TD) dairies as well as in atmospheric (background) air

Microbial group/genus/species	Sample origin						
	CD		TD		Atmospheric background		
	Air	Surface	Air	Surface			
Gram-positive cocci	<i>Aerococcus</i> spp.	nd ^{b)}	nd	0.5	nd	nd	
	<i>Kocuria kristinae</i>	1.2	nd	nd	nd	nd	
	<i>Kocuria rosea</i>	nd	nd	nd	nd	6.3	
	<i>Kocuria</i> spp.	nd	0.5	nd	nd	nd	
	<i>Lactococcus lactis</i> ssp. <i>lactis</i> ^G	4.2	nd	4.4	4.0	nd	
	<i>Leuconostoc mesenteroides</i> ssp. <i>cremoris</i> ^G	nd	5.0	nd	2.0	nd	
	<i>Micrococcus luteus</i>	nd	nd	2.7	nd	nd	
	<i>Micrococcus</i> spp.	8.4	1.5	3.0	0.5	13.6	
	<i>Staphylococcus aureus</i> *	nd	2.7	4.5	5.1	nd	
	<i>Staphylococcus caprae</i>	nd	nd	1.6	nd	nd	
	<i>Staphylococcus capitis</i>	nd	1.4	nd	nd	nd	
	<i>Staphylococcus chromogenes</i>	nd	nd	1.0	nd	nd	
	<i>Staphylococcus haemolyticus</i>	1.3	1.6	nd	nd	nd	
	<i>Staphylococcus saccharolyticus</i>	nd	nd	3.5	nd	nd	
	<i>Staphylococcus sciuri</i>	nd	1.0	nd	1.0	nd	
	<i>Staphylococcus simulans</i>	0.5	nd	0.5	nd	0.6	
	<i>Staphylococcus</i> spp.	1.5	nd	0.5	nd	2.0	
	<i>Staphylococcus xylosum</i>	1.0	1.8	0.5	1.0	0.7	
	<i>Streptococcus intermedius</i> *	nd	7.0	4.1	4.2	nd	
	<i>Streptococcus thermophilus</i> ^G	6.7	7.9	11.5	10.5	nd	
Gram-positive bacilli	<i>Bacillus cereus</i>	4.5	nd	6.9	2.2	2.0	
	<i>Bacillus circulans</i>	nd	nd	1.7	nd	nd	
	<i>Bacillus licheniformis</i>	1.0	nd	1.6	0.4	nd	
	<i>Bacillus megaterium</i>	nd	nd	2.3	nd	nd	
	<i>Bacillus mycoides</i>	nd	nd	0.5	nd	nd	
	<i>Bacillus pumilus</i>	1.5	nd	0.5	nd	3.5	
	<i>Bacillus</i> spp.	2.6	nd	1.2	nd	15.8	
	<i>Clostridium perfringens</i> *	nd	0.8	nd	1.9	nd	
	Non-sporing Gram-positive rods	<i>Bifidobacterium</i> spp. ^G	17.8	17.1	15.6	5.0	nd
		<i>Lactobacillus buchneri</i> ^G	nd	nd	nd	2.5	nd
<i>Lactobacillus casei</i> ssp. <i>paracasei</i> ^G		nd	nd	nd	nd	nd	
<i>Lactobacillus curvatus</i> ^G		nd	nd	3.5	18.5	nd	
<i>Lactobacillus delbrueckii</i> ssp. <i>delbrueckii</i> ^G		3.4	20.5	nd	5.5	nd	
<i>Lactobacillus fermentum</i> ^G		nd	13.2	nd	11.2	nd	
<i>Lactobacillus lactis</i> ssp. <i>cremoris</i> ^G		nd	nd	5.0	nd	nd	
<i>Lactobacillus lactis</i> ssp. <i>lactis</i> ^G		6.9	nd	4.7	nd	nd	
<i>Lactobacillus plantarum</i> ^G		nd	nd	0.5	nd	nd	
<i>Lactobacillus paracasei</i> ssp. <i>paracasei</i> ^G		9.7	9.0	nd	14.3	nd	
<i>Lactobacillus</i> spp. ^G		8.5	nd	1.2	nd	nd	
<i>Microbacterium</i> spp.		nd	1.0	nd	0.5	nd	
Ma	<i>Actinomyces</i> spp.*	0.4	nd	nd	nd	nd	
	<i>Streptomyces</i> spp.*	nd	nd	1.0	nd	nd	

Table 4. Percentage contribution (%) of bacteria and fungi to total microbiota isolated from the air and surface samples collected in commercial (CD) and traditional (TD) dairies as well as in atmospheric (background) air (Continuation)

Microbial group/genus/species	Sample origin				
	CD		TD		Atmospheric background
	Air	Surface	Air	Surface	
<i>Acremonium</i> spp.	1.0	nd	0.5	nd	3.0
<i>Acremonium strictum</i>	0.9	0.5	0.7	0.2	0.5
<i>Aspergillus calidoustus</i>	nd	nd	0.2	0.2	6.5
<i>Aspergillus niger</i>	nd	nd	0.5	nd	nd
<i>Aspergillus parasiticus</i>	nd	nd	nd	0.5	nd
<i>Aspergillus</i> spp.	nd	nd	0.5	nd	11.2
<i>Fusarium oxysporum</i>	0.7	nd	nd	nd	nd
<i>Fusarium poae</i>	0.9	nd	nd	nd	nd
<i>Fusarium solani</i>	nd	nd	0.5	nd	nd
<i>Fusarium sporotrichoides</i>	nd	nd	nd	nd	2.5
<i>Fusarium</i> spp.	0.5	nd	0.9	nd	4.9
<i>Fusarium verticilloides</i>	0.5	nd	nd	nd	nd
<i>Penicillium aurantiogriseum</i>	nd	nd	1.2	nd	nd
<i>Penicillium brevicompactum</i>	nd	nd	nd	nd	10.8
<i>Penicillium citrinum</i>	nd	nd	0.5	nd	nd
<i>Penicillium commune</i>	nd	0.5	nd	nd	nd
<i>Penicillium crustosum</i>	1.2	nd	0.8	nd	nd
<i>Penicillium discolor</i>	2.5	nd	nd	nd	3.7
<i>Penicillium glaucum</i>	3.5	nd	nd	nd	2.3
<i>Penicillium griseofulvum</i>	nd	nd	0.2	nd	nd
<i>Penicillium nalgiovense</i>	nd	nd	0.2	nd	2.0
<i>Penicillium nordicum</i>	nd	nd	2.2	nd	1.2
<i>Penicillium</i> spp.	4.0	nd	3.5	nd	5.4
<i>Rhizopus nigricans</i>	0.5	nd	nd	nd	nd
<i>Scopulariopsis candida</i>	nd	1.0	nd	0.5	nd
<i>Wallemia sebi</i>	nd	1.5	nd	2.3	nd
<i>Candida albicans</i> *	nd	1.3	nd	1.0	nd
<i>Candida famata</i>	nd	0.5	nd	0.3	nd
<i>Candida glabrata</i>	nd	nd	nd	0.8	nd
<i>Candida rugosa</i>	nd	0.5	nd	0.5	nd
<i>Cryptococcus albidus</i>	nd	nd	0.2	0.5	nd
<i>Cryptococcus laurentii</i>	nd	nd	0.2	nd	nd
<i>Cryptococcus</i> spp.	nd	nd	0.5	nd	nd
<i>Debaryomyces hansenii</i>	nd	0.2	nd	0.5	nd
<i>Geotrichum candidum</i>	2.7	0.5	1.3	0.5	1.5
<i>Rhodotorula glutinis</i>	nd	0.5	nd	nd	nd
<i>Rhodotorula mucilaginosa</i>	nd	nd	0.9	nd	nd
<i>Yarrowia lipolytica</i>	nd	1.0	nd	1.9	nd

^{a)}nd – not detected; Ma – mesophilic actinomycetes; ^g– species with GRAS status; * – biological agents from risk group 2 (according to the classifications in Directive 2000/54/EC and in Ordinance of the Polish Minister of Health).

Table 5. Concentrations of bacteria and fungi (CFU/cm²) on surfaces in commercial (CD) and traditional (TD) dairies

Type of surface	Bacteria				Fungi			
	CD		TD		CD		TD	
	Median	Range	Median	Range	Median	Range	Median	Range
Worktops in milk reception	21.8	19.3–27.4	50.1	49.8–50.3	1.2	0.9–1.4	4.6	4.2–4.7
Surfaces of tanks in milk storage	22.7	21.9–23.2	40.1	39.8–42.5	0.5	0.1–1.0	2.0	0.9–4.0
Worktops in cheese production	49.7	43.2–58.2	60.4	55.1–67.1	2.7	1.6–3.0	2.2	1.5–2.6
Worktops in cream and butter production	3.7	1.6–5.2	0.2	0.1–0.3	0	0–0	0	0–0
Conveyor belts in packaging area	1.0	0.4–1.2	0.7	0.2–1.0	0	0–0	0	0–0

Table 6. Microbial pathogens isolated from the air and surfaces in commercial (CD) and traditional (TD) dairies

Microorganism	CD		TD	
	Bioaerosol	Surface	Bioaerosol	Surface
<i>Staphylococcus aureus</i>	nd ^{a)}	Worktops in milk reception	Milk reception Cheese production Cream and butter production	Worktops in milk reception Worktops in cheese production Worktops in cream and butter production
<i>Streptococcus intermedius</i>	nd	Worktops in milk reception	Cheese production	Worktops in milk reception Worktops in cheese production Worktops in cream and butter production
<i>Clostridium perfringens</i>	nd	Worktops in milk reception	nd	Worktops in milk reception
<i>Streptomyces</i> spp.	nd	nd	Milk reception	nd
<i>Actinomyces</i> spp.	Milk reception	nd	nd	nd
<i>Candida albicans</i>	nd	Worktops in milk reception Tanks surfaces in milk storage	Cheese production	Worktops in milk reception Surfaces of tanks in milk storage Worktops in cheese production

^{a)}nd – not detected

isolated from surfaces in TD. The comparison between dairies revealed that the studied surfaces were, to the same extent, contaminated with moulds and yeasts.

As in the case of air samples, biological agents from risk group 2 were also detected on the tested surfaces. In CD, *Staphylococcus aureus* constituted 2.7%, *Streptococcus intermedius* 7.0%, *Clostridium perfringens* 0.8%, and *Candida albicans* 1.3% of total surface microbiota. In TD, the contribution of *Staphylococcus aureus*, *Streptococcus intermedius*, *Clostridium perfringens*, and *Candida albicans* to total surface microbiota, was equal 5.1%, 4.2%, 1.9%, and 1.0%, respectively. Also for these microorganisms, molecular analysis positively verified their sequence similarities at the level of 99–100%, with the sequences deposited in the GenBank database.

Bacterial and fungal pathogens in dairies. Strains belonging to the group 2 according to Directive 2000/54/EC and Ordinance of the Minister of Health and the workplaces where they were detected are listed in Table 6. *Staphylococcus aureus*, *S. intermedius*, *Streptomyces* spp., and *C. albicans* were isolated from bioaerosols samples collected in TD, while *Actinomyces* spp. was present in bioaerosol samples collected in CD. All these strains were primarily detected within milk reception zones (CD, TD), cheese production (TD), and cream and butter production (TD) areas. *Staphylococcus aureus*, *S. intermedius*, *C. perfringens*, and *C. albicans* contaminated surfaces in both CD and TD, and were isolated from worktops in milk reception, tanks surfaces in milk storage, worktops in cheese as well as in cream and butter production areas.

DISCUSSION

This study confirmed that bacteria and fungi are commonly present at workplaces in dairies. The higher concentrations of bacteria and fungi in the air and on surfaces were obtained in TD compared to CD. Regardless the level of automation of dairy production in CD and TD, the most microbiologically polluted workplaces were located within milk reception and milk storage areas. Poor microbial quality in small scale dairies with manual processing has also been observed by other authors [37]. Air quality studies conducted by Salustiano et al. [38] revealed that the highest bacterial concentrations were observed in milk reception (313 CFU/m³) and cheese production areas (381 CFU/m³), while the highest concentrations of fungi were found in butter and cream (410 CFU/m³) and cheese production sections (342 CFU/m³). According to Belestioids et al. [39], the concentration of fungal aerosol in the yoghurt production area was on the level of 70 CFU/m³. Against this background, the results regarding bacteria concentration in the air of milk reception area obtained in the current study were higher (in CD equal to 860 CFU/m³; in TD equal to 14639 CFU/m³) than those observed by Salustiano et al. [38]; however, the results regarding bacterial and fungal concentrations in the air at workplaces of other studied dairy sections were lower.

Generally, higher concentrations of microorganisms in the air in this study, with statistically significant differences in case of bacteria, were observed in traditional dairies than in commercial ones. It is known that environmental factors may affect bioaerosols concentration and diversity [40], thus there are several factors that may contribute to air pollution in these dairy processing areas. Among them, the processed material (especially raw milk), manufacturing

practices and microclimate parameters play an essential role in determining the concentration of microorganisms [41]. For example, Salustiano et al. [38] noticed that air humidity affects the fungal concentration in bioaerosols; however, in the current study neither humidity nor temperature significantly influenced the airborne bacterial and fungal concentrations ($p>0.05$). High concentrations of bacteria and fungi in the air of the milk reception and milk storage areas observed in this study may result from the high intensity movement of a large number of milk suppliers, local farmers, and dairy workers. Moreover, the frequently performed cleaning of milk tanks using pressurized water may also lead to aerosolization of raw milk residues along with microorganisms growing on them. As described in the Guide to Environmental Microbiological Testing for the Food Industry [42], most food manufacturing processes involve one or more steps that effectively kill pathogenic bacteria; however, the manufacturing of some dairy products may not involve such steps in the production cycle. Thus, dairy workers may be exposed to microbiological agents throughout all production process.

Despite the fact that a few hundred million workers around the world are exposed to airborne biological agents, there are still no widely accepted threshold limit values for microbial contaminants. Among the reasons for this are: difficulties in determination of the dose–response relationship for most biological agents, inability to identify individual microbial species responsible for adverse health outcomes, individual susceptibility to a specific biological agent of exposed person, a lack of standardization of sampling methods and experimental procedures, and a still insufficient database regarding environmental and occupational concentrations of biological agents. Being aware of all these scarcities, the Polish Expert Group for Biological Agents of the Interdepartmental Commission for Maximum Admissible Concentrations and Intensities for Agents Harmful to Health in the Working Environment at the Central Institute for Labour Protection–National Research Institute (CIOP–PIB), proposed threshold limit values (TLV) for microbiological agents in the air of occupational and non-occupational environments, taking ‘environmental factors’ into account (Tab. 7) [43, 44, 45]. Several other authors also proposed air quality standards dedicated to different dairy processing areas (Tab. 8) [46, 47].

Based on the above, from the workers’ safety point of view in both CD and TD in the current study, the threshold limit values for microbiological agents in the air were not

Table 7. Threshold limit values for bioaerosols proposed by the Polish Expert Group for Biological Agents of the Interdepartmental Commission for Maximum Admissible Concentrations and Intensities for Agents Harmful to Health in the Working Environment (Górny et al., 2008; Górny et al., 2007; Pośniak, 2018)

Microbial agent	Workplaces polluted with organic dust	Residential and public utility premises
Mesophilic bacteria	100,000 CFU ^a /m ³	5,000 CFU/m ³
Gram-negative bacteria	20,000 CFU/m ³	200 CFU/m ³
Thermophilic actinomycetes	20,000 CFU/m ³	200 CFU/m ³
Fungi	50,000 CFU/m ³	5,000 CFU/m ³
Microbial agents from risk groups 3 and 4	0 CFU/m ³	0 CFU/m ³
Bacterial endotoxins	200 ng/m ³ (2,000 EU ^b /m ³)	5 ng/m ³ (50 EU/m ³)

^aCFU – colony forming unit; ^bEU – endotoxin unit

Table 8. Suggested air quality standards for various processing areas in dairy industry (Mostert and Jooste, 2002; Luck and Gavron, 1990)

Processing area	Bacteria		Yeasts and molds	
	Satisfactory	Unsatisfactory	Satisfactory	Unsatisfactory
Fermented milk products, cottage cheese	<150 CFU ^a /m ³	>1500 CFU/m ³	<50 CFU/m ³	>1000 CFU/m ³
Milk and cream	<150 CFU/m ³	>1500 CFU/m ³	<50 CFU/m ³	>1000 CFU/m ³
Butter	<100 CFU/m ³	>1000 CFU/m ³	<50 CFU/m ³	>1000 CFU/m ³
Powdered milk	<200 CFU/m ³	>2000 CFU/m ³	<100 CFU/m ³	>1000 CFU/m ³
Ripened cheese	<200 CFU/m ³	>2000 CFU/m ³	<100 CFU/m ³	>1000 CFU/m ³

^aCFU – colony forming unit

exceeded. However, taking into account the suggested hygienic standards for the dairy industry, the obtained results were not satisfactory. The concentrations of bacteria in the air at almost all workplaces in TD (excluding the packaging area) and within the milk reception and milk storage areas in CD were above the proposed TLVs. The concentrations of fungi at all workplaces in both types of studied dairies were also not satisfactory. Airborne fungi may be responsible for dairy product contamination and their spoilage, and (even in low concentrations) can cause many adverse health effects, including respiratory disorders, e.g. allergic diseases, bronchial asthma, and allergic pulmonary alveolitis, as well as skin allergies or irritations [48].

Raw milk may also contaminate processing area surfaces and, as such, become a secondary emission source for the bioaerosols [38]. Moreover, the attachment of microorganisms to surfaces and processing equipment may lead to biofilm formation which can affect the safety and quality of food products [49]. In the studied dairies, the most bacterially contaminated surfaces were worktops in cheese production and milk reception areas, as well as the surface of tanks in milk storage. In the case of fungi, the most contaminated surfaces in CD were the worktops in cheese production, while in TD the worktops in milk reception.

There are several proposals regarding the cleanliness of surfaces in the dairy industry. According to Godlewska [50], permissible contamination for dairy equipment surfaces should not exceed 100 CFU/cm². Other sources indicate that for cleaned contact surfaces, the satisfactory level of total viable microorganisms should be less than 80 CFU/cm² [37, 51]. Taking into account standards indicated by the Public Health Laboratory Service [51], the levels of contamination of tested surfaces were not exceeded. Nevertheless, dermal contact with some microorganisms, e.g. LABs and strains belonging to the risk group 2, according to Directive 2000/54/EC may play an important role in skin irritation and other adverse health outcomes among exposed workers. It should also be emphasized that according to microbiological guidelines, *S. aureus* belonging to the risk group 2 according to Directive 2000/54/EC, detected in the current study on worktops in milk reception (CD, TD), worktops in cheese production (TD) and worktops in cream and butter production areas (TD), should be absent on all food-contact surfaces [37, 52].

In this study, a total of 43 microbial species belonging to 14 genera were isolated from the dairies. Gram-positive cocci and non-sporing Gram-positive rods were the predominant groups, both in bioaerosol and on surfaces in CD and TD. Higher concentrations of bacteria and fungi were mainly

observed in TD, where manual processing of raw milk was the method of its technological handling. Gram-positive cocci, isolated in this study, are usually a part of the human and animal microbiota, but may also be common in the environment. The species from *Staphylococcus*, *Streptococcus*, *Leuconostoc*, and *Lactococcus* genera are especially, characteristic for the working environment where raw milk is usually processed [53]. Two pathogens from this group, *Staphylococcus aureus* and *Streptococcus intermedius*, present in bioaerosols from TD and on surfaces from CD and TD may cause skin, subcutaneous, soft tissue and systemic infections, as well as inflammation of the lungs, endocarditis, and necrotizing fasciitis [54, 55, 56]. Both these strain, probably originated from the processed milk, are responsible for mastitis in cattle and are often present in raw milk [57, 58]. *Staphylococcus aureus* has also been frequently isolated from cheese production lines [59]. According to Schlegelová et al. [60] staphylococci are present quite often in dairies on technical equipment, and the results obtained in the current study confirmed this observation. This study also indicates the fact that contamination of food contact surfaces was relatively high even after their sanitation process. It is worth mentioning that the equipment surfaces in dairy facilities may be contaminated with staphylococci closely related to multi-resistant strains persisting in biofilm communities, and this information should be kept in mind when efficient hygienic procedures have to be introduced into the production processes [37, 52].

In turn, non-sporing Gram-positive rods belonging to the *Bifidobacterium* and *Lactobacillus* genera are probiotic bacteria, also known as lactic acid bacteria. They are desirable in the dairy production processes and their occurrence is a natural phenomenon in this environment [61, 62]. Many of the identified LABs have GRAS status [20, 63]; however, according to Zeilfelder et al. [21], they may cause irritations and generate specific immune responses among exposed workers. Hence, their presence in the occupational environment should be always taken into account during risk exposure assessment.

Other Gram-positive bacteria, including the *Bacillus* species, as well as mesophilic actinomycetes (like those from *Streptomyces* and *Actinomyces* genera), are commonly present in the environment, mainly in soil and plants. They are probably transmitted to the workplaces with outdoor air through the ventilation system and/or by humans on their cloths and footwear [41]. However, some other isolated species from this group, e.g. anaerobic *Clostridium perfringens* and aerobic *Bacillus cereus*, may come from processed milk and have been already detected in milk tanks [64, 65, 66]. Bacterial strains belonging to *Bacillus*, *Leuconostoc*, *Kocuria*, and *Staphylococcus* genera have been also isolated in dairy plants by other authors and seem to be typical for microbiota of these types of processing areas [67].

Regarding the mycobiota of the studied dairies, the identified moulds represent typical environmental species and in the observed concentrations should not pose a risk to workers with a properly functioning immune system. In dairies, mould contaminants are derived mainly from the atmospheric air, while yeasts usually originate from milk. Regardless of animal species, raw milk generally may contain fungi (usually more yeast cells than fungal conidia) in concentrations between 10^3 – 10^5 CFU/mL [68]. *Candida albicans* shows a high prevalence in milk from cows in

Poland. In term of yeasts, however, some pathogenic strains were present both in the air and on surfaces from studied dairies. *Candida albicans* and other *Candida* species are by far the most common causes of bovine mastitis [69]. *C. albicans* is also a major fungal pathogen for humans that may cause mucosal infections and, in certain groups of vulnerable individuals, severe, life-threatening bloodstream infections and subsequent infections of the internal organs [70]. Strains belonging to species *Cryptococcus*, *Geotrichum*, *Rhodotorula*, *Debaryomyces*, and *Yarrowia* occur in milk and dairy products as non-starter yeasts. They may make important contributions to the flavour and texture of specialty cheeses [10]; however, the health impact of most of the non-starter yeasts and moulds remains unknown.

An important part of safety work management is proper health risk assessment. Identification and classification of isolated microorganisms to a proper risk group, depending on the ability of microorganism to causing infection, the possibility of spreading and prophylaxis methods and effective treatment, should be taken into account in the initial stage of occupational risk assessment. It should be noted that in case of harmful biological agents, occupational activities with bioaerosols production (e.g. cleaning milk tanks), work with high concentrations of substances (e.g. adding starter cultures) or manual activities with the risk of injury, may increase the level of infection risk for workers.

CONCLUSIONS

Bioaerosols and surfaces in the occupational environment in dairies contain a high diversity of microorganisms, regardless of the type of dairy plant. This study revealed that, compared to commercial dairies, a higher bacterial and fungal contamination of the processing environment was observed in traditional dairies, where the manual processing of raw milk was the method of its technological handling. In both TD and CD, the noted microbial air and surface concentrations did not exceed the threshold limit values proposals; however, from the point of view of microbial quality of processing areas, the measured contamination levels were not satisfactory in most cases. What is important, is that the strains belonging to risk group 2 according to Directive 2000/54/EC were present in bioaerosol and surface samples in both types of studied dairies and, as such, may significantly influence the health status of dairy workers and the quality of milk products. Thus, the control of microbial air and surface contamination should be routinely carried out as a part of hygienic quality assessment within dairies. Moreover, identification and classification of isolated microorganisms to a proper risk group should be an essential part of occupational risk assessment. The introduction and improvement of adequate hygienic practices, such as hand washing and cleaning and disinfection procedures, should directly enhance the microbial quality of the processing environment, and translate into the improvement of safety of both dairy workers and consumers.

Conflict of interest

The authors declare that they have no conflict of interest.

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